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Synthesis of Novel Cationic Lipids with a Guanidine Group. Cationic Lipids 3[1]

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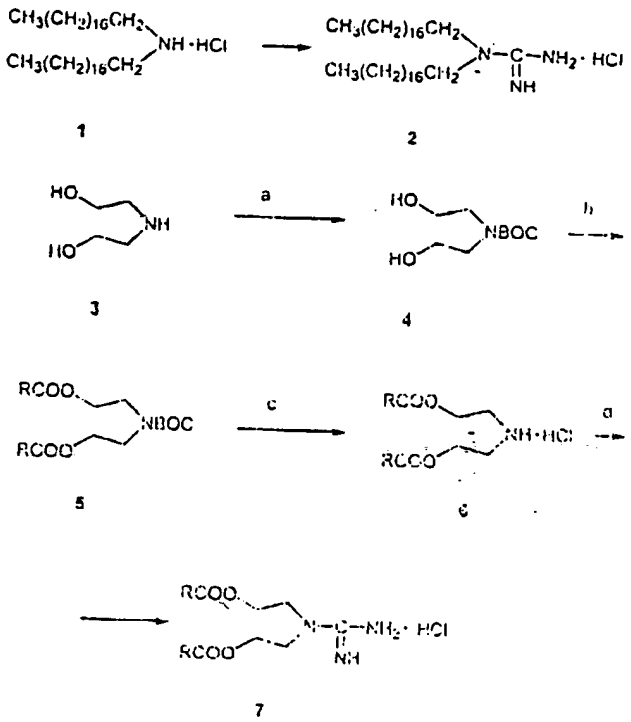
Abstract: Potentially cell biodegradable cationic lipids having a strongly basic guanidine group have been prepared by the reaction of lipophilic derivatives of diethanolamine with cyanamide.

Cationic lipids are valuable materials for the preparation of cationic liposomes for drug delivery and gene transfection.[2] Cationic lipids bear a positively charged head group, which, as a rule consists of a single or multiple nitrogen atoms with variable extents of substitution.[3] Other nitrogen containing functionalities also could be involved in the formation of a positively charged head group.[4] Because of the strongly basic properties (pK_a 12[5]) of guanidine, its lipophilic derivatives potentially could serve as a valuable source of cationic lipids.[6] The method most often used for preparation of guanidine derivatives involves the reaction of amine hydrochlorides with cyanamide.[7] A number of other guanylating reagents were reported, including O-methylisourea hydrogen sulfate,[8] S-alkylisothiouronium salts,[9] salts of 1*H*-pyrazole-1-carboxamidines,[10] and 1*H*-pyrazole-1-[*N,N'*-bis(*tert*-butoxycarbonyl)]-carboxamidine.[11]

For the purpose of successful in vivo gene therapy, it will be essential for the cationic lipid to be biodegradable by cells. Otherwise, accumulation of unmetabolized lipid may cause toxic cell effects. Replacement of the non-metabolizable long-chain aliphatic amine 1 with moieties containing fatty acids linked to the head group by readily metabolized ester bonds seems highly desirable. From this point of view, diethanolamine 3 is a quite suitable starting material (Scheme). After protection of the amino group with the acid labile *tert*-butoxycarbonyl (BOC) protective group, acylation with the appropriate fatty acid chloride, and removal of the BOC group by treatment with 4M HCl in dioxane, the resulting hydrochloride 6 was reacted with cyanamide in *n*-butanol at 120°C, conditions found to be optimal.



Product	RCO	Yield %
7a	$CH_3(CH_2)_{12}CO$	59
7b	$CH_3(CH_2)_{14}CO$	90
7c	$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO$	55

Reagents and conditions: a. BOC_2O/CH_2Cl_2, RT. b. RCOCl, NEt_3/CH_2Cl_2, 0°C. c. HCl/dioxane, RT. d, H_2NCN/n-BuOH, 120°.

Scheme. Preparation of Lipophilic Guanidines

Typical experimental procedure for the preparation of lipophilic guanidines 7[12]:

To 2.0 g (0.003 mol) of hydrochloride 6c were added 0.25 g (0.006 mol) of cyanamide and 5.0 ml of n-butanol. The resulting mixture was stirred at 120°C for 1.5 hr. Completion of the reaction was monitored by TLC (Merck F_{254} silica gel on glass baked plates, developing solvent: 10% MeOH/90% $CHCl_3$, spots were visualized by spraying the TLC plate with 4% ethanol solution of phosphomolybdic acid, and heating it on a hotplate at 150° C for 10 min). The cooled mixture was diluted with 100 ml of $CHCl_3$, washed twice with 5% aqueous NaCl:MeOH/1:1, dried over anhydrous Na_2SO_4, and evaporated on a rotavapor. The residue was purified on a silica gel column using 1-10% MeOH in $CHCl_3$ as the eluant. After evaporation, 1.11g (55% yield) of guanidine 7c was obtained as a colorless waxy solid. Some selected data: R_f 0.43; ^1H NMR (300 Mhz, $CDCl_3$) δ 0.88 (m, 2CH_3CH$_2$, 6H), 1.26, 1.28 (each s, 40 H, 2 $(CH_2)_6$ and 2 $(CH_2)_4$); 1.59 (m, 4 H, 2 CH_2CH$_2$CO); 2.00 (m, 8 H, 2 CH_2CH=CHCH_2); 2.31 (m, 4 H, 2 CH$_2$CH_2CO); 3.76 (m, 4 H, 2 NCH_2CH$_2$O); 4.29 (m, 4 H, 2 NCH$_2$CH_2O); 5.35 (m, 4 H, 2 CH=CH); 7.54 (br s, 3H, C(=NH)NH$_2$); ^{13}C NMR (75.5 Mhz, $CDCl_3$) δ 173.66, 158.55, 130.01, 129.68, 61.46, 48.89, 33.99, 31.89, 29.71, 29.52, 29.31, 29.19, 29.12, 27.21, 24.71, 22.67, 14.11. Anal. calcd.: C, 69.09; H, 11.05; N, 5.90. Found: C, 69.25; H, 10.91; N, 6.05.

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